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Predicting Biotransformations in the Subsurface: Relationship between the ATP (Adenosine triphosphate) Content of Subsurface Material and the Capacity of Subsurface Organisms to Degrade Toluene

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PREDICTING BIOTRANSFORMATIONS IN THE SUBSURFACE: RELATIONSHIP BETWEEN THE ATP CONTENT OF SUBSURFACE MATERIAL AND THE CAPACITY OF SUBSURFACE ORGANISMS TO DEGRADE TOLUENE

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16. ABSTRACT

Deeper subsurface material was collected in a manner that prevented contamination by surface microorganisms. This material was analyzed for ATP content, and for its capacity to degrade toluene, a common organic contaminant of ground water originating from release of petroleum products. In material from a pristine site near Lula, OK where the permanent water table was at 4m the ATP content of material from 1.5, 2.9, and 4.6m was 1.2, 1.1, and 0.14 ng/g respectively. Material from all three depths degraded toluene rapidly. Material from just below the water table at 7.5m at a near-pristine site in Conroe, TX, had an ATP content of only 0.017 mg/g, and could not degrade toluene. All four samples had essentially the same number of cells as determined by Acridine Orange Direct Count. Studies are in progress to determine whether deeper material from the Lula site with a low ATP content (0.03 ng/g) can degrade toluene in order to determine if ATP is a candidate index-compound to predict active biotransformation of industrial contaminants.

17. KEY WORDS AND DOCUMENT ANALYSIS

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INTRODUCTION

Petroleum-derived hydrocarbons are important organic contaminants of ground water. Toluene and other alkyl benzenes are among the water-soluble components of gasoline and diesel fuel and are released into ground water from spills of these materials. They are also commonly encountered in leachate from municipal landfills and hazardous waste disposal sites. Chlorobenzene is also commonly ecountered in ground water polluted by hazardous waste sites.

Estimates of the rate of biodegradation of these compounds in subsurface materials is needed for damage assessment at existing sites, for intelligent permitting of activities that may release these materials to the subsurface, and for the most effective design of clean-up and remediation activities (Dunlap et al. 1984). At present these estimates are only available through expensive, tedious, and lengthy microcosm studies (Wilson et al. 1983). There is a need for an index to predict biotransformation that can be determined rapidly and at low cost. The total number of cells as determined by direct examination is not a good index because the numbers are surprisingly uniform from one site to another and at different depths in the same site, eventhough biodegradation potential varies widely in the same material (Wilson et al. 1983)

This study evaluates ATP (Adenosine triphosphate) as a candidate index because this compound is a integral part of the energy metabolism of all terrestial life, and because a sensative array is available for ATP in sub-]surface material (Webster et al. 1983).

MATERIALS AND METHODS

Sampling

All work was done on samples that were acquired in a maneuver that included contamination from surface microorganisms (Wilson et al. 1983) with the exception of a single datum noted in Table 1, all samples analyzed for ATP were extracted in the field to preclude changes in ATP content resulting for disturbances associated with sampling.

Microcosms

Core material was stirred with additions of sterile distilled water until it became a slurry. This slurry was aseptically transferred to 40 ml screw-cap test tubes; then 1.0 ml of a sterile solution of the organic compounds was added to each tube. Then the tubes were sealed and the organic compounds mixed through the slurry with a vortex mixer. After the appropriate incubation, the microcosms were analyzed by diluting the pore water and analyzing the diluted pore water by the purge-and-trap method (see Wilson et al. 1983 for details). Trichloroethylene was added to the material from Lula as an internal standard.

Precautions were taken to insure that the pore water of the slurries contained at least 0.5 mg/l of oxygen. The microcosms were incubated in the dark at the ambient temperature (25°C and 17°C for material from Conroe, TX and Lula, OK respectively). Portions of core material was autoclaved prior to construction of some of the microcosms in order to distinguish disappearance due to biotransformation from abiotic processes.

RESULTS

The ATP content of subsurface material from the two study sites has been reported previously (Webster et al. 1985). This paper compares the ATP content of the material to the rate of biodegradation of toluene and other organic pollutants by microbes in the subsurface material. The behavior of toluene and chlorobenzene in pristine subsurface material was studied in unconsolidated material from the margin of the flood plain of a small river near Lula, OK (Fig. 1). As are many riverine deposits, the unconsolidated section was very complex. The section was arbitrarily divided into the following discrete layers. First, a fine silty-clay subsoil extending to the permanent water table near 2.5 m. This material has strong structure with many cracks and root channels. Second, a layer of fine sand about 0.5 m thick just below the permanent water table. This material has no structure, is very plastic, and contains enough clay to make it impermeable. Third, a mixture of coarse sands and gravels extending from 3 to 4.5 m, then a layer of uniform sands extending to 5.6 m. Both these layers are very transmissive. Fourth, below the sand is a layer of gravels down to 7.2 m. These gravels are in a matrix of sand and clay and are impermeable. The bed rock is a massive olive-green clay. The ATP content of the subsoil and the fine sand just below the water table was very similar. Both rapidly degraded toluene. Chlorobenzene degradation was detectable, but was much slower. The ATP content of the transmissive uniform sands was an order of magnitude lower, and the rate of degradation of toluene was reduced at least six-fold. Surprisingly, the rate of degradation of chlorobenzene actually increased. In the impermeable grounds the ATP content was still lower, and there was no detectable degradation of toluene or chlorobenzene.

The relationship between ATP content and toluene degradation in polluted subsurface material was studied at a site in Conroe, TX. A disposal lagoon for wood-creosoting process wastes produced a plume of water containing a number of polynuclear aromatic hydrocarbons and polyheterocyclic hydrocarbons at individual concentrations of 800 to 200 µg/liter and benzene, toluene, m-xylene, o-xylene, and p-xylene at individual concentrations of 30 to 15 µg/liter. The present water table is 7.6 to 8.5 in. deep. The pollution is moving through a layer of transmissive sand 2.0 to 2.5 m thick that lies above an impermeable layer of clay.

At the margin of the plume the contaminated water is mixed with oxygenated pristine water, which allows microbes in the aquifer to degrade the hydrocarbons and renovate the water in the plume. This renovated water can be recognized because it still contains elevated concentrations of chloride representative of the plume (Wilson et al. 1985).

In pristine material from this site collected just below the water table, the ATP content is low, and there is no evidence of biodegradation of toluene, benzene, o-xylene or m-xylene (Table 1). Rates of disappearance of these compounds in pristine subsurface material was not different from the rates of disappearance in autoclaved subsurface material. Material was sampled from two sites of a well (5A) that penetrated a region of the aquifer containing renovated water. In material that was further away from the source of pollution, ATP content was low and there was no statistically significant evidence for degradation of the alkyl benzenes. However, this particular assay had a low sensitivity; rates would have had to exceed 10 $year^{-1}$ to be statistically significant. In material sampled closer to the source of

pollution, the ATP content was similar to the ATP content of the uniform transmissive sand at Lula, OK and materials from both sites exhibited similar rates of degradation of toluene.

CONCLUSIONS

The capacity of subsurface material to degrade toluene was related to its ATP content. There was no detectable capacity of indigenous subsurface microorganisms to degrade toluene, other alkyul benzenes, or chlorobenzene whenever the ATP content of the subsurface material was at or below 0.05 ng/g.

The variability in ATP content and biodegradation potential was surprisingly high at the Lula, OK site. They varied over two orders of magnitude over a depth interval of only 4 m.